IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	: 10/561,793	Confirmation No.:	3642
Applicant	: Guy VANCANNEYT, *et al.*		
Filed	: December 21, 2005		
Title	: METHODS AND MEANS FOR DELAYING SEED SHATTERING IN PLANTS		
TC/Art Unit	: 1638		
Examiner:	: Li ZHENG		
Docket No.	: 58764.000055		
Customer No.	: **21967**		

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Johan Botterman, Ph.D. declare that:

1. I am a citizen of Belgium residing at Het Wijngaardeke 5, Zevergem, Belgium 9840.

2. I received a degree of engineering for chemistry and agricultural industries from the University of Ghent, Belgium, in 1981, and a Doctor of Philosophy degree in agricultural sciences from the University of Ghent, in 1986. I have published more than forty scientific papers and obtained over fifteen U.S. patents in the field of plant biology and biotechnology. A listing of these papers and patents, as well as a description of my professional experience are provided in my *curriculum vitae*, attached herewith as **Exhibit A.**

3. I have been employed by Bayer BioScience N.V. ("Bayer"), or its predecessors (e.g., Aventis CropScience) since 1986. I am currently the Head of Product Research at Bayer. Bayer is a co-assignee of the above-identified application ("the '793 application").

4. Based on my academic training and professional experience, I consider myself to be a person of at least ordinary skill in the art of plant biology and biotechnology, and I was such a person prior to June 23, 2003, the foreign priority date claimed in the '793 application.

The Claimed Invention

5. I have read the '793 application. I understand this application discloses and claims the use of a dsRNA construct based on part of the nucleotide sequence of the *Arabidopsis* IND1 gene other than a bHLH domain coding region to increase podshatter resistance in *Brassica* species (e.g., *Brassica napus*) to the extent that the pods still can be opened along the dehiscence zone by applying limited physical forces. *See, e.g.*, '793 application, Example 2B.

The Office Action

6. I have reviewed the Office Action mailed September 7, 2007 ("Office Action"), and the Final Office Action mailed July 23, 2008 ("Final Office Action"). I understand that the U.S. Patent and Trademark Office has rejected claims 1, 2, 15-17, 24, and 27, as allegedly being obvious over U.S. Patent No. 7,135,621 ("Yanofsky") or U.S. Patent No. 6,998,517 ("Liljegren"), in combination with Smith et al. (Nature, 407:319-320, 2000) ("Smith").

7. I understand that the Examiner asserts that a person of ordinary skill in the art would have found it obvious to modify the gene silencing vector of Yanofsky or Liljegren by replacing the antisense fragment disclosed in these references with an inverted repeat structure taught by Smith. I also understand that the Examiner contends that the skilled artisan would have been motivated to do so, given the teaching of Smith, that the modifications increase the efficiency of gene silencing compared to antisense constructs. *See* Office Action, page 15.

8. I further understand that the Examiner asserts that a dsRNA construct comprising any fragment over 23 base pairs of the *Arabidopsis* IND1 gene other than a bHLH domain coding region is considered an obvious choice for gene silencing, absent

evidence that using such dsRNA would generate unexpected results. *See* Final Office Action, page 3.

The Cited References

9. Yanofsky and Liljegren disclose the identification of the INDEHISCENT1 (IND1) gene from *Arabidopsis thaliana*, the identification and analysis of *Arabidopsis* plants containing a mutation in the IND1 gene which results in indehiscent fruits, as well as promoter/enhancer::GUS fusions of the IND1 gene. When the IND1::GUS fusions were introduced in *Arabidopsis* wild-type plants, about 25% of the transgenic lines failed to express significant GUS activity and displayed an indehiscent phenotype. The references suggest that the most likely explanation of these results is that the IND1::GUS fusions, as well as the endogenous IND1 gene were co-suppressed. *See* Yanofsky, col. 27, lines 19-29; Liljegren, col. 27, lines 7-17.

10. The Yanofsky application is a continuation-in-part of the Liljegren application. Yanofsky discloses the nucleotide sequence of two IND1 orthologs from *Brassica napus*. I have been informed, however, that this information was added at the time of filing the Yanofsky application (i.e., June 18, 2004). This date is later than the foreign priority date claimed in the '793 application (i.e., June 23, 2003). Accordingly, I have been informed that Yanofsky's disclosure of the two IND1 orthologs from *Brassica napus* is not prior art to the '793 application.

11. The Examiner asserts that Smith teaches (1) a DNA construct that produces hairpin loop type of dsRNA (hpRNA) with functional (i.e. splicable) intron as spacer enhances silencing efficiency; and (2) the modifications that help to align the complementary arms of the hairpin and promote the formation of a duplex could increase the efficiency of gene silencing. I agree with the Examiner's characterization of Smith. However, I fail to see the relevance of Smith's teaching in the context of the claimed invention. The claimed invention concerns the *weakening* of gene silencing of the IND1 gene, whereas Smith concerns *enhancing* gene silencing in general. Therefore, one of ordinary skill in the art would not be motivated (or have a reason) to modify Yanofsky's (or Liljegren's) method to achieve the claimed methods by replacing Yanofsky's (or

Liljegren's) antisense fragment with Smith's hairpin structure. Rather, one of ordinary skill in the art would actually be discouraged from combining Yanofsky (or Liljegren) and Smith to achieve the claimed methods.

Knowledge Of The Skill Artisan

12. Prior to June 23, 2003, the foreign priority date claimed in the '793 application, the nucleotide sequences of IND1 orthologs from *Brassica* plants were not publicly known. Accordingly, a person skilled in the art could not have made any prediction of the degree of sequence identity between the *Arabidopsis* IND1 gene and the orthologs from *Brassica napus* plants or any subparts thereof such as the bHLH domain coding region.

13. Furthermore, prior to June 23, 2004, the filing date of the '793 application, a person of ordinary skill in the art would have been aware of the (probable) co-suppression results reported in Yanofsky and Liljegren (discussed above), which resulted in indehiscent phenotypes. The skilled artisan would also have known that silencing of the IND gene in *Arabidopsis thaliana* using so-called dsRNA silencing techniques resulted in almost complete podshatter resistance, and that 98% of the transgenic *Arabidopsis* lines developed siliques, which did not open along the valve suture and could only be opened by applying considerable pressure to the valves. *See, e.g.*, '793 application, ¶ 10 (as published in US 2006/248612).

14. As discussed in paragraph 11, it is my opinion that one of ordinary skill in the art would not have combined Yanofsky (or Liljegren) with Smith. Even assuming that one of ordinary skill in the art would have combined the teaching of Yanofsky (or Liljegren) with the teaching of Smith and (1) constructed a chimeric gene encoding a dsRNA based on part of the *Arabidopsis* IND1 nucleotide sequence; and (2) introduced the chimeric gene into an oilseed rape plant, then the skilled person would, in my opinion, only expect two possible outcomes:

a. the nucleotide sequences of the dsRNA based on *Arabidopsis* IND1 and of the endogenous IND1 *Brassica* orthologs are sufficiently identical to allow gene-

silencing to be established and consequently an indehiscent phenotype as observed in *Arabidopsis* would be expected (see paragraph 13 above); or

b. the nucleotide sequences of the dsRNA based on *Arabidopsis* IND1 and of the endogenous IND1 *Brassica* orthologs are too divergent for any gene-silencing phenomenon to be established and no effect on *Brassica* IND1 gene expression and dehiscence phenotype are to be expected.

15. In view of the knowledge of one of ordinary skill in the art, at the time the '793 application was filed, that gene-silencing typically involves sequences of 21-24 consecutive nucleotides, and in view of the relatively close taxonomic relationship between *Arabidopsis* and *Brassica*, it is my opinion that a person of ordinary skill in the art would probably have favored option a) above.

16. A person of ordinary skill in the art would not have been in a position to predict that the use of dsRNA based on parts of the nucleotide sequence of IND1 from *Arabidopsis* would result in *Brassica* plants with an intermediate degree of gene-silencing sufficient to prevent seed shattering, but not so extensive as to prevent the normal opening along the dehiscence zone with the application of moderate physical forces common in agricultural practices such as the use of combine harvesters.

Conclusion

17. Based on the above information, it is my opinion that prior to the instant invention it was unexpected that a chimeric gene encoding a dsRNA based on part of the nucleotide sequence of the *Arabidopsis* IND1 gene not including a bHLH domain coding region would result in an increased pod shatter resistance combined with an agronomically relevant threshability of the pods in *Brassica* species such as *B. napus, Brassica junceae*, and *Brassica campestris*

I declare that all statements made herein are based on personal knowledge or upon information and belief and are believed to be true; and further that the statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Dated: April 20, 2009

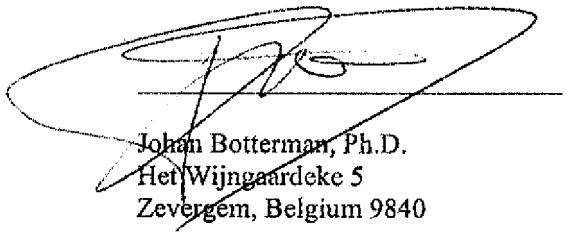

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Exhibit A

CURRICULUM VITAE

Name: Johan H. Botterman
Address: Het Wijngaardeke 5
9840 Zevergem
Date of birth: 30-05-1958

Education:

1981	University of Ghent – Faculty of agriculture – engineer for chemistry and agricultural industries
1981-1982	Grant from I.W.O.N.L.
1982-1986	Grant from N.F.W.O.
1986	University of Ghent – Faculty of Agriculture – Ph. D. in Agricultural Sciences

Working experience:

1986 - 1989	Plant Genetic Systems N.V. - Project Leader Molecular Biology
1989 - 1992	Plant Genetic Systems N.V. - Product Development Manager
1993 - 1996	Plant Genetic Systems N.V. - Research Manager
1997 - 1999	Hoechst Schering Agrevo GmbH – Head of Biotechnology Research – Brassica oilseeds and vegetables
1999 - 2002	Aventis Crop Science – Research Manager – Brassica oilseeds Member of the Directoire of RhoBio SA and Genoplante SAS
2002 - 2004	Bayer BioScience N.V. - Product Research Manager Health and Canola
2005 - 2007	Bayer BioScience N.V. - Product Research Manager Health and Bio-Economy crops
2007 -	Bayer BioScience N.V. - Head of Product Research

A. Publications:

1:
Vancanneyt G, Dubald M, Schröder W, Peters J, Botterman J.
A case study for plant-made pharmaceuticals comparing different plant expression and production systems.
Methods Mol Biol. 2009;483:209-21.

2:
Nadai M, Bally J, Vitel M, Job C, Tissot G, Botterman J, Dubald M.
High-level expression of active human alpha1-antitrypsin in transgenic tobacco chloroplasts.
Transgenic Res. 2009; 18(2):173-83.

3:
Tissot G, Canard H, Nadai M, Martone A, Botterman J, Dubald M.
Translocation of aprotinin, a therapeutic protease inhibitor, into the thylakoid lumen of genetically engineered tobacco chloroplasts.
Plant Biotechnol J. 2008; 6(3):309-20.

4:
Giritch A, Marillonnet S, Engler C, van Eldik G, Botterman J, Klimyuk V, Gleba Y.
Rapid high-yield expression of full-size IgG antibodies in plants coinfected with noncompeting viral vectors.
Proc Natl Acad Sci U S A. 2006; 103(40):14701-6.

5:
Field B, Cardon G, Traka M, Botterman J, Vancanneyt G, Mithen R.
Glucosinolate and amino acid biosynthesis in Arabidopsis.
Plant Physiol. 2004; 135(2):828-39.

6:
Van Breusegem F, Slooten L, Stassart JM, Moens T, Botterman J, Van Montagu M, Inzé D.
Overproduction of Arabidopsis thaliana FeSOD confers oxidative stress tolerance to transgenic maize.
Plant Cell Physiol. 1999; 40(5):515-23.

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Down-regulation of cinnamyl alcohol dehydrogenase in transgenic alfalfa (Medicago sativa L.) and the effect on lignin composition and digestibility.
Plant Mol Biol. 1999; 39(3):437-47.

8:
Van Breusegem F, Kushnir S, Slooten L, Bauw G, Botterman J, Van Montagu M,Inzé D.
Processing of a chimeric protein in chloroplasts is different in transgenic maize and tobacco plants.
Plant Mol Biol. 1998; 38(3):491-6.

9:
Van Breusegem F, Slooten L, Stassart JM, Botterman J, Moens T, Van Montagu M, Inze D
Effects of overproduction of tobacco MnSOD in maize chloroplasts on foliar tolerance to cold and oxidative stress.
Journal of Experimental Botany 1999; 50(330): 71-78

10:
D'Halluin K, Botterman J
"The Use of Agrobacterium for Plant Genetic Engineering"
in "The Rhizobiaceae: Molecular Biology of Model Plant-Associated Bacteria" by J. J. Hooykaas, P. Spaink. Publisher: Springer-Verlag New York, LLC, August 1998

11:
Wehrmann A, Van Vliet A, Opsomer C, Botterman J, Schulz A.
The similarities of bar and pat gene products make them equally applicable for plant engineers.
Nat Biotechnol. 1996;14(10):1274-8.

12:
Strittmatter G, Janssens J, Opsomer C, Botterman J
Inhibition of Fungal Disease Development in Plants by Engineering Controlled Cell Death
Bio/Technology 1995; 13: 1085 - 1089

13:
McKersie BD, Chen Y, de Beus M, Bowley SR, Bowler C, Inzé D, D'Halluin K, Botterman J.
Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (Medicago sativa L.).
Plant Physiol. 1993;103(4):1155-63.

14:
de Oliveira DE, Franco LO, Simoens C, Seurinck J, Coppieters J, Botterman J, Van Montagu M.
Inflorescence-specific genes from Arabidopsis thaliana encoding glycine-rich proteins.
Plant J. 1993;3(4):495-507.

15: Dhalluin K, Bossut M, Bonne E, Mazur B, Leemans J, Botterman J (1992)
Transformation of sugar beet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants.
BioTechnology 1992; 10 : 309-314

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Denecke J, De Rycke R, Botterman J.
Plant and mammalian sorting signals for protein retention in the endoplasmic reticulum contain a conserved epitope.
EMBO J. 1992;11(6):2345-55.

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D'Halluin K, De Block M, Denecke J, Janssens J, Leemans J, Reynaerts A, Botterman J.
The bar gene as selectable and screenable marker in plant engineering.
Methods Enzymol. 1992;216:415-26.

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Expression of a bacterial lysine decarboxylase gene and transport of the protein into chloroplasts of transgenic tobacco.
Plant Mol Biol. 1991;17(3):475-86.

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Denecke J, Goldman MH, Demolder J, Seurinck J, Botterman J.
The tobacco luminal binding protein is encoded by a multigene family.
Plant Cell. 1991; 3(9):1025-35.

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Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J, Sybesma C, Van Montagu M, Inzé D.
Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants.
EMBO J. 1991;10(7):1723-32.

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Characterization of phosphinothricin acetyltransferase and C-terminal enzymatically active fusion proteins.
Gene. 199;102(1):33-7.

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Differential expression of five Arabidopsis genes encoding glycine-rich proteins.
Plant Cell. 1990;2(5):427-36.

23:
Denecke J, Botterman J, Deblaere R.
Protein secretion in plant cells can occur via a default pathway.
Plant Cell. 1990;2(1):51-9.

24:
D'Halluin K, Botterman J, De Greef W
Engineering of Herbicide-Resistant Alfalfa and Evaluation under Field Conditions
Crop Sci 1990; 30:866-871

25:
Negrutiu I, Dewulf J, Pietrzak M, Botterman J, Rietveld E, Wurzer-Figurelli EM, De Y, Jacobs M
Hybrid genes in the analysis of transformation conditions: II. Transient expression vs stable transformation—analysis of parameters influencing gene expression levels and transformation efficiency
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Bio/Technology 1989; 7: 929–932.

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Plant molecular farming: production of peptides with high-added value
Med. Fac. Landbouww. Rijksuniv. Gent (Belgium) 1989; 54(4): 1249-1251

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Angenon G, Uotila J, Kurkela SA, Teeri TH, Botterman J, Van Montagu M, Depicker A.
Expression of dicistronic transcriptional units in transgenic tobacco.
Mol Cell Biol. 1989;9(12):5676-84.

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Transgenic expression of two marker genes under the control of an *Arabidopsis rbcS* promoter: Sequences encoding the Rubisco transit peptide increase expression levels
Molecular and General Genetics 1989; 218(1): 78-86

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Field testing of insect and herbicide resistant crops.
Vortr. Pflanzenzüchtg. 1989; 16:455-461.

31:
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Methods Mol. Cell 1989; 1: 19-27.

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Peleman J, Boerjan W, Engler G, Seurinck J, Botterman J, Alliotte T, Van Montagu M, Inzé D.
Strong cellular preference in the expression of a housekeeping gene of Arabidopsis thaliana encoding S-adenosylmethionine synthetase.
Plant Cell. 1989;1(1):81-93.

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Bio/Technology 1989; 7: 61 - 64

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Botterman J, Leemans J
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Biotechnology and Genetic Engineering Reviews (United Kingdom) 1988; 6: 321-340

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Botterman J, Leemans J.
Engineering herbicide resistance in plants.
Trends Genet. 1988;4(8):219-22.

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Improvement of agricultural crops by genetic engineering
Med. Fac. Landbouww. Rijksuniv. Gent (Belgium) 1988; 53(4): 1695-1699.

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High-level expression of genes under control of cro translation initiation signals in Escherichia coli.
Journal of biotechnology 1987; 6(1): 71-81.

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Botterman J, Zabeau M.
A standardized vector system for manipulation and enhanced expression of genes in Escherichia coli.
DNA. 1987;6(6):583-91.

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Fermentation and recovery of the EcoRI restriction enzyme with a genetically modified Escherichia coli strain.
Biotechnol Bioeng. 1985;27(9):1320-7.

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Spriet JA, Botterman J
Correction factors for the dynamic measurement of the volumetric mass-transfer coefficient
J. Chem. Tech. Biotechnol. 1984; 34(4): 137 - 153

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Spriet JA, Botterman J, Buyser DR, De Visscher PL, Vandamme EJ.
A Computer-aided noninterfering on-line technique for monitoring oxygen-transfer characteristics during fermentation processes. Biotechnol Bioeng. 1982;24(7):1605-21.

B. U.S. Patent Applications and Patents:

1:
US2005/0142122 Brassica plant resistant to the fungus Leptosphaeria maculans (blackleg)
Diederichsen E, Laga B, Botterman J

2:
US 7,501,559 Prevention of Bt resistance development
Van Mellaert H, Botterman J, Van Rie J, Joos H

3:
US 7,112,665 Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants
Leemans J, Botterman J, De Block M, Thompson C, Mouva R

4:
US 6,855,873 Recombinant plant expressing non-competitively binding Bt insecticidal crystal proteins
Van Mellaert H, Botterman J, Van Rie J, Joos H

5:
US 6,797,861 Seed shattering
Ulvskov P, Child R, Van Onckelen H, Prinsen E, Borkhardt B, Sander L, Petersen M, Poulsen GB, Botterman J

6:
US 6,420,628 Seed shattering
Ulvskov P, Child R, Van Onckelen H, Prinsen E, Borkhardt B, Sander L, Petersen M, Poulsen GB, Botterman J

7:
US 6,344,602 Method to obtain male sterile plants
Michiels F, Botterman J, Cornelissen M

8:
US 6,172,281 Recombinant plant expressing non-competitively binding BT insecticidal crystal proteins
Van Mellaert H, Botterman J, Van Rie J, Joos H

9:
US 6,025,546 Method to obtain male sterile plants
Michiels F, Botterman J, Cornelissen M

10:
US 5,908,970 Recombinant plant expressing non-competitively binding Bt insecticidal crystal proteins
Van Mellaert H, Botterman J, Van Rie J, Joos H

11:
US 5,866,784 Recombinant plant expressing non-competitively binding insecticidal crystal proteins
Van Mellaert H, Botterman J, Van Rie J, Joos H

12:
US 5,648,477 Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants
Leemans J, Botterman J, De Block M, Thompson C, Mouva R

13:
US 5,646,024 Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants
Leemans J, Botterman J, De Block M, Thompson C, Mouva R

14:
US 5,623,067 Seed-specific promoter region
Vandekerckhove JS, Krebbers E, Botterman J, Leemans J

15:
US 5,561,236 Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants
Leemans J, Botterman J, De Block M, Thompson C, Mouva R

16:
US 5,487,991 Process for the production of biologically active peptide via the expression of modified storage seed protein genes in transgenic plants
Vandekerckhove JS, Krebbers E, Botterman J, Leemans J

17:
US 5,460,963 Plants transformed with a DNA sequence from Bacillus thuringiensis lethal to Lepidoptera
Botterman J, Peferoen M, Hofte H, Joos H